CORRELATION BETWEEN PHARMACOKINETIC PROPERTIES AND ^{15}N-NMR AND ^{13}C-NMR CHEMICAL SHIFTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS

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ABSTRACT

In the current study, angiotensin converting enzyme inhibitor molecules (enalapril, perindopril, ramipril and benazepril) were studied using ^{15}N-nuclear magnetic resonance techniques like insensitive nuclei enhanced by polarization technique and heteronuclear multiple bond correlation. The chemical shift data and different pharmacokinetic and physicochemical properties of the molecules like $pK_{a,}$ half-life, time taken to reach maximum concentration *in vivo*, were compared and found to exhibit a good linear relationship. Further, a similar comparative study of the same parameters was performed using ^{13}C chemical shifts of the molecules. Molecular docking studies were also performed to understand the structure of the enzyme bound to the ligand. This is perhaps the first report of ^{15}N-nuclear magnetic resonance studies of the molecules as well as studies correlating the relationships between ^{15}N chemical shifts, pharmacokinetics and physicochemical properties in molecules which could be used for the prediction of properties for which experimental data is currently not available.

Keywords: ^{15}N-Nuclear magnetic resonance, benazepril, enalapril, perindopril, ramipril structure activity relationship studies.

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors are therapeutic agents used for the treatment of hypertension and congestive heart failure. ACE, also known as kininase II, is present in a membrane-bound form in the endothelial cells; it is a bivalent dipeptidyl carboxyl metallopeptidase in the epithelial or neuroepithelial cells. It is also present in the brain, circulatory blood and in a number of body fluids in a soluble form[1]. ACE comprises of two different isoforms; somatic and germinal/testicular ACE. The somatic ACE consists of two identical catalytic domains and a cytoplasmic tail; it is a large protein of 150-180 kDa found in epithelial and neural cells. The germinal ACE comprises of a single catalytic domain that communicates with COOH-terminal domain of the somatic ACE; it is a small protein of 100-110 kDa found in developing spermatids and mature sperm cells only[2,3].

The Renin-Angiotensin-Aldosterone system (RAAS) is an important humoral mechanism for regulating blood pressure and electrolyte homeostasis in blood circulation. Renin, a proteolytic enzyme released from the renal arterioles, hydrolyzes angiotensinogen and α-globulin, produced by the liver. The product of this hydrolysis is a decapeptide, angiotensin I, which has little, if any, biological activity. Angiotensin I is readily cleaved to an octapeptide, angiotensin II by angiotensin converting enzyme (ACE). Angiotensin II is a powerful vasoconstrictor that causes an increase in blood pressure. The octapeptide also triggers the release of a steroid hormone aldosterone, which causes an increase in blood pressure by promoting the excretion of potassium ions and retention of sodium ions and water. Aminopeptidase-A converts angiotensin II to angiotensin III, which also causes vasoconstriction and promotes release of aldosterone. Further, ACE also catalyzes the hydrolysis of the potent vasodilator nonapeptide, bradykinin. Due to hydrolysis, bradykinin loses its vasodilator activity[4,5]. Hence, ACE is responsible for the generation of two potent vasoconstrictors, angiotensin II and angiotensin III and release of another steroid hormone aldosterone, which causes sodium ion and water retention. Additionally, it destroys bradykinin, a vasodilator agent. The results of all these actions of ACE cause an increase in blood pressure. Thus, inhibiting ACE can result in reduction of blood pressure by shutting down the hypertensive mechanisms caused by ACE activity. The RAAS system is summarized in Fig. 1.

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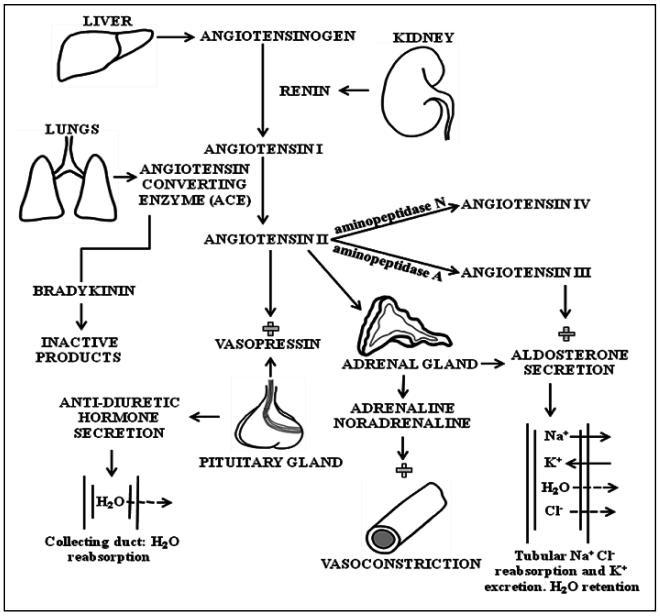

Fig. 1: Mechanism by which ACE causes hypertension

ACE is an important target for the design of antihypertensive agents. ACE inhibitors can be divided into three main classes based on the group that binds to the zinc component of the ACE active site. All ACE inhibitors contain one of the three functional groups: the sulfhydryl group, the dicarboxyl group or the phosphinyl group[6]. The chemical structure, bioavailability, potency, plasma half-life and the route of elimination differ between different ACE inhibitors.

Captopril was the first ACE inhibitor to be developed and marketed[4]. Captopril contains a sulfhydryl as the main functional group that binds to the zinc ligand. The phosphinyl functional group is present in fosinopril. The largest class of ACE inhibitors is the carboxyalkyl dipeptide containing molecules, which include enalapril, ramipril, perindopril, lisinopril, quinapril, benazepril, trandolapril, imidapril and cilazapril.

Drugs having a common mechanism of action can be clustered into the same therapeutic class with similar efficacies of functioning. However, differences in the chemical structure can result in differences in physicochemical and pharmacokinetic properties which, in turn, can have an effect on the pharmacological properties. These differences are often clinically relevant, for example, in deciding dosing schedules and drug-turnover time. Frequently, data from systematic experimental investigation of physico-chemical and pharmacokinetic properties are sparse and many a times not available. Hence, it is important to have reliable prediction methods for the same.

Nuclear Magnetic Resonance (NMR) investigates the atomic arrangement in molecules. NMR spectrum represents the local environment around the particular nucleus that is being studied. The chemical shift of nuclei in similar environments will be similar even if they are in different molecules. Hence, NMR chemical shift values reflect detailed structural information of the molecule. This information can be useful in predicting the physicochemical or pharmacokinetic properties of molecules.

In this study, we have investigated the relationship between the different properties and ^{15}N chemical shifts as well as ^{13}C chemical shifts of molecules belonging to the carboxyalkyl dipeptide class. This is perhaps the first report of ^{15}N NMR studies for enalapril, ramipril, perindopril and benazepril. Further, to understand the structure-activity relationship of the potent ACE inhibitors, molecular docking studies were also performed.

MATERIALS AND METHODS

Materials

Drug samples of enalapril, ramipril, perindopril and benazepril were obtained as gift samples in pure form from Quality Control Laboratories (QCL) of Dr. Reddy's, Mylan and Laurus Labs.

^{15}N NMR experiments

All NMR experiments were carried out using Bruker Avance 400 MHz instrument operating at 40 MHz for ^{15}N nucleus at a temperature of 298.2 K. The chemical shifts are reported with reference to liquid ammonia. Due to the low gyromagnetic ratio ($\gamma = -27.126 \times 10^6\ T^{-1}s^{-1}$) the sensitivity of ^{15}N nucleus in NMR is very low. The low natural abundance of the ^{15}N isotope (0.37%) further adds to the problem of detection of the ^{15}N nucleus. We were unable to detect any signals using direct detection or 1D experiment like Insensitive Nuclei Enhanced by Polarization Technique (INEPT). Hence, all the molecules were studied using ^{1}H-^{15}N Heteronuclear Multiple Bond Correlation (HMBC) experiments. The solvent used was dimethyl sulfoxide (DMSO-d6).

^{13}C NMR data

The ^{13}C chemical shifts of seven molecules, enalapril[7], perindopril[8], ramipril[9], benazepril[10], lisinopril[11], trandolapril[12] and imidapril[13] were obtained from the literature.

In silico docking studies

All seven (benazepril, imidapril, enalapril, perindopril, trandolapril, lisinopril, ramipril) ACE inhibitors were docked onto Angiotensin converting enzyme (ACE) (PDB:

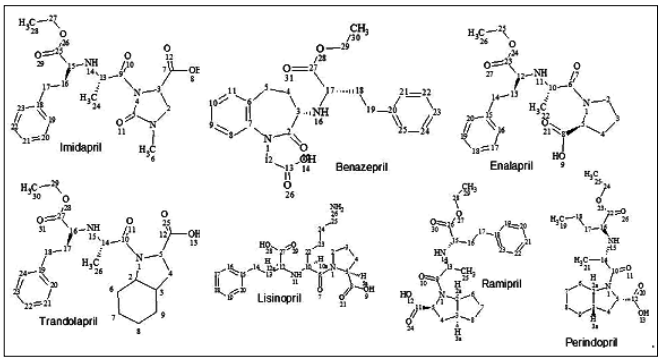

Fig. 2: Structures of ACE Inhibitor molecules with the numbering scheme

1O8A) using molecular docking software PatchDock[14,15]. Captopril was used as the standard reference molecule. The structures of the ligands were constructed using Dundee PRODRG server[16]. The precise location of the binding site and the potentiality of the ligand to bind to the active site were determined using an automated docking software, Molegro Virtual Docker 2008, version 3.2.1 (Molegro ApS, Aarhus, Denmark) that utilizes the MolDock docking engine[17].

RESULTS AND DISCUSSION

The structures of the ACE inhibitor molecules studied, along with the atom numbering scheme are shown in Fig. 2.

^{15}N NMR studies for any of the molecules have apparently not been reported earlier. This is possibly the first report of ^{15}N NMR studies for enalapril, ramipril, perindopril and benazepril. The projection of the ^{15}N channel of the HMBC spectra for the four molecules, enalapril, perindopril, ramipril and benazepril are shown in Figs. 3-6. The chemical shift assignments are shown in Table I.

Table I: ^{15}N chemical shift assignments of ACE inhibitors

Molecule	^{15}N Chemical Shift (ppm)	
	Amino Nitrogen	Ring Nitrogen
Enalapril	57	132.39
Perindopril	53.32	152.32
Ramipril	49.35	142.59
Benazepril	44.81	126.94

The ACE inhibitor pharmacophore of enalapril analogues can be described as N-carboxymethyl-L-alanyl-L-proline[18], which is shown in Fig. 7.

The structures of enalapril, ramipril, trandolapril and imidapril vary only with respect to the ring (R3) at the dipeptide's carboxyl terminus. Benazepril has a similar structure, except for the proline ring that has been incorporated into a seven membered ring. Perindopril differs from enalapril both in the R1 and R3 regions. Lisinpopril does not contain an ester at the side-chain carboxyl group, and has an aminobutyl $H_2N(CH_2)_4$ for the side chain methyl group.

Different methods were described in the literature[19] for utilizing ^{13}C chemical shifts in the development of

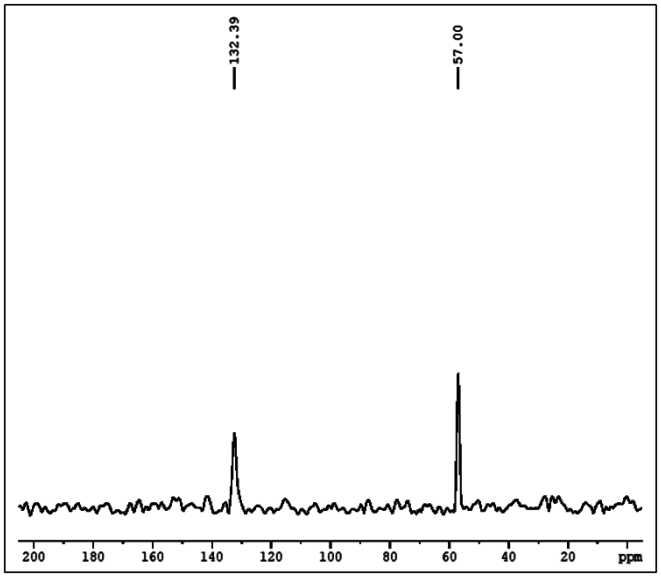

Fig. 3: Projection of ^{15}N channel of the ^{1}H-^{15}N HMBC NMR spectrum of enalapril

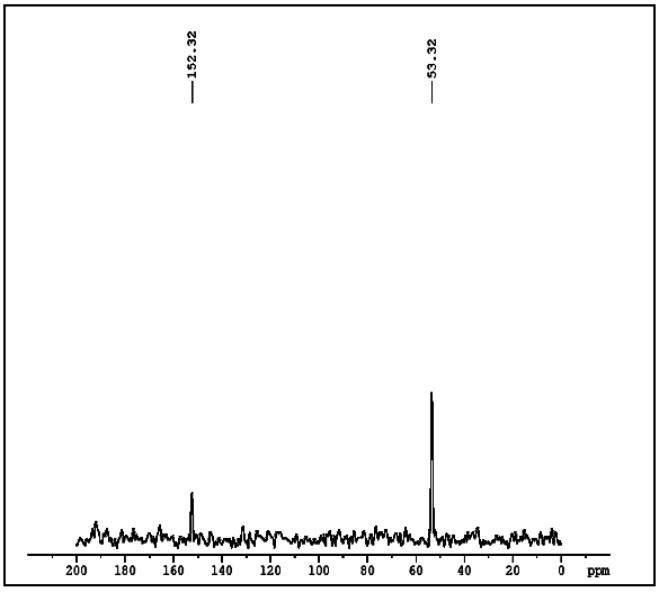

Fig. 4: Projection of ^{15}N channel of the ^{1}H-^{15}N HMBC NMR spectrum of perindopril

quantitative structure activity relationship models. The methods include studying the sum of the chemical shifts and the study of the chemical shift of a particular atom

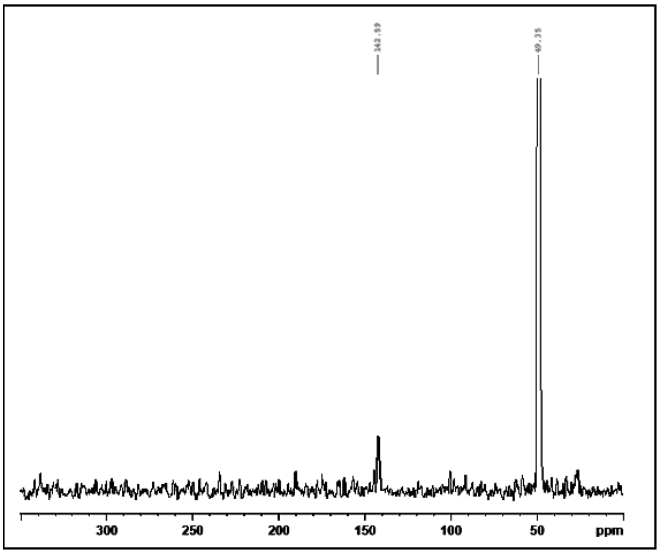

Fig 5: Projection of ^{15}N channel of the ^{1}H-^{15}N HMBC NMR spectrum of ramipril

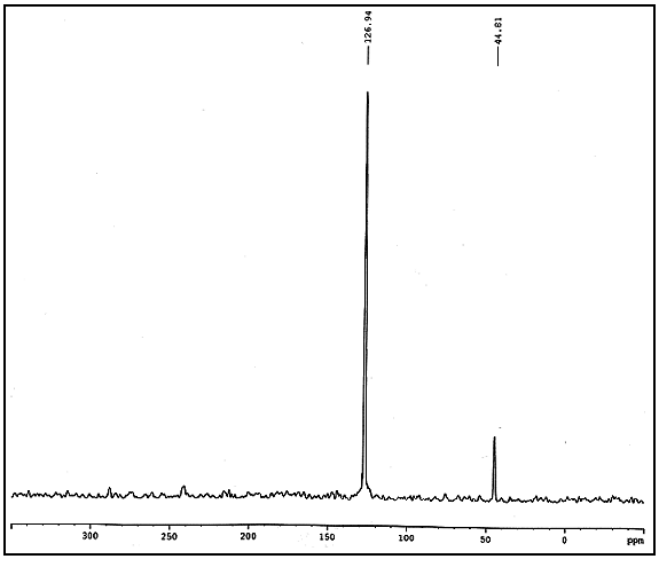

Fig 6: Projection of ^{15}N channel of the ^{1}H-^{15}N HMBC NMR spectrum of benazepril

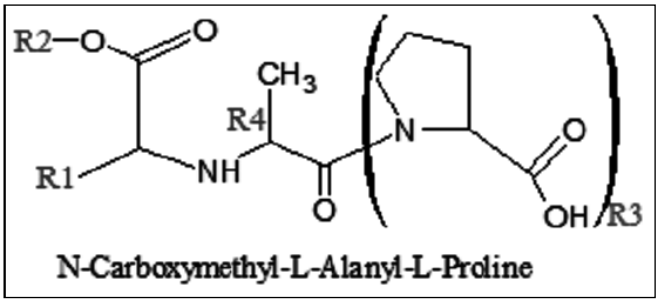

Fig 7: The ACE pharmacophore for enalapril analogues with the different moieties indicated

common to all molecules. In this study, the chemical shifts of the nitrogens common to all the molecules and their correlation with different properties were studied. In the case of ^{13}C chemical shifts, the sum of chemical shifts of the three moieties, R1, R2 and R3 and the chemical shift of the atom at R4 position were studied. These values are summarized in Table II.

Table II: Sum of ^{13}C chemical shifts of R1, R2, R3, R4 moieties

Molecule	Sum of ^{13}C chemical shifts (ppm)			
	R1	R2	R3	R4
Enalapril	852.46	78.32	339.26	56.04
Perindopril	68.5	75	462	52.8
Ramipril	854.73	77.48	478.14	53.94
Benazepril	835.9	40.5	1116.6	59
Lisinopril	867.36	-	356.57	61.56
Trandolapril	846.96	77.1	473.4	48.2
Imidapril	871.75	75.97	420.71	54.1

The carboxylic group attached to the R2, which is usually an ethyl, except in the case of lisinopril, is the important group binding to the zinc binding site and the R1 moiety binds to an auxiliary binding site. The side-chain-NH forms hydrogen bond with the enzyme and is considered important for inhibitory activity. The cyclic nature of the substituents in R3 position provides for steric hindrance to protect the amide bond from hydrolysis by amidases. The data for the different physicochemical and pharmacokinetic properties of the molecules were collected from the literature[20,21] and are summarized in Table III.

Table III: Pharmacokinetic and physicochemical properties of the selected molecules

Molecule	Half-life (h)	T_{max} (h)	log P	pK_a (acid)	pK_a (base)
Enalapril	11	4	0.07	3.74	5.15
Perindopril	9	4	2.6	3.78	5.33
Ramipril	12	3	2.9	3.74	5.15
Benazepril	21	1.5	3.3	3.04	4.74
Lisinopril	13	7	-1.01	3.66	6.07
Trandolapril	6	4	3.5	3.74	5.15
Imidapril	1.7	2	3.08	3.49	5.22

Correlation coefficient, r, is a measure of the relationship between two sets of variables. It varies between-1 and +1; -1 indicating a strong negative

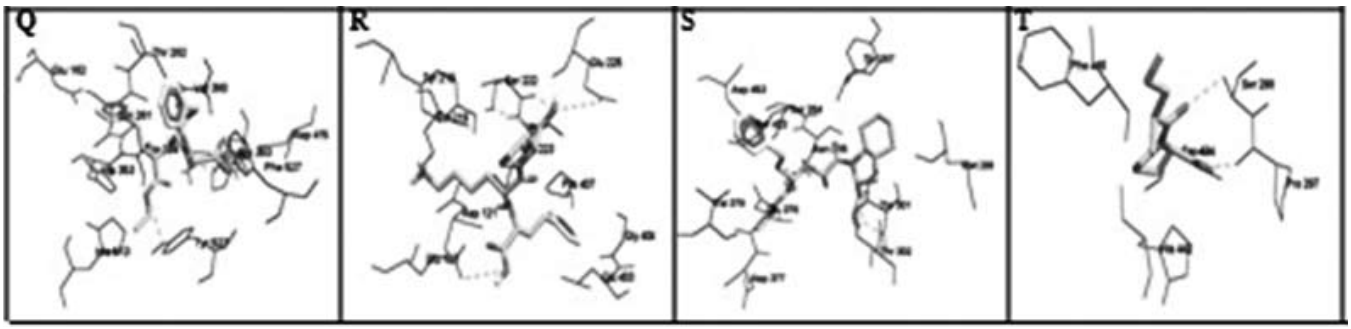

Fig 8: Molecular docking studies: Q-ACE and Benazepril, R-ACE and Lisinopril, S-ACE and Trandolapril, T-ACE and Captopril

relationship and +1 indicating a strong positive relationship. A value of zero indicates the lack of any relationship between the two variables. The linear regression equation models the relationship between two sets of variables. The goodness of fit for the data is measured by R-squared value, which lies between 0 and 100. The value indicates how close the data are to regression line. A high R-squared value indicates a good fit of the data to the modeled equation.

The correlation between ^{15}N-NMR and ^{13}C-NMR chemical shifts and different physicochemical and pharmacokinetic properties of ACE Inhibitors are discussed in the following sections.

Correlation between pK_a and ^{15}N-NMR and ^{13}C-NMR chemical shifts

ACE inhibitors exhibit at least two pK_a values. They contain a carboxylic group attached to the proline or R3 moiety, which can be ionized and a basic amine group that can be protonated. Lisinopril contains a second ionizable carboxylic group. The sum of ^{15}N chemical shifts (^{15}N-Sum) exhibits an excellent correlation with basic pK_a (r = 0.98) which can be modelled as follows:

pK_a (basic) = 0.0176 (±0.0025) (^{15}N-Sum) + 1.756 (±0.482) - Eq.1

n = 4, S.E = 0.0611, R^2 = 0.960

The sum of ^{13}C chemical shifts of the R2 moiety (^{13}C-R2), which is in the vicinity of the amino nitrogen, also exhibits a good positive correlation with the basic pK_a (r = 0.91), which can be modeled by eq2.

pK_a (basic) = 0.012 (±0.003) (^{13}C-R2) + (±0.203) - Eq.2

n = 6, S.E = 0.0938, R^2 = 0.824

Thus, chemical shift values of the nitrogens and as well as the chemical shift of the carbons of the ethyl group in the vicinity of the amino nitrogen that undergoes protonation reflect the variation in the basic pK_a of the ACE inhibitors.

The acidic pK_a also exhibits a linear relationship with the sum of ^{15}N chemical shifts (r = 0.884). The sum of ^{13}C chemical shifts of the R3 ring moiety containing the ionizable carboxyl group correlates negatively with pK_a (acid) with a correlation coefficient of -0.89. The sum of ^{13}C chemical shifts of the R2 ring moiety also shows significant correlation with pK_a (acid). The following equations represent the relationships:

pK_a (acid) = 0.022 (± 0.008) (^{15}N-Sum) - 0.728 (± 1.61) -Eq.3

n = 4, S.E = 0.204, R^2 = 0.781

pK_a (acid) = 0.018 (± 0.003) (^{13}C-R2) + 2.305 (± 0.249) -Eq.4

n = 6, S.E = 0.115, R^2 = 0.873

pK_a (acid) = -0.001 (± 0.0002) (^{13}C-R3) + 4.058 (± 0.114) - Eq.5

n = 6, S.E = 0.130, R^2 = 0.798

As mentioned earlier, the structures of all the seven molecules are very similar, differing only at the R3 position, except in the case of perindopril and lisinopril. The strong correlation between pK_a values and chemical shifts shows that the structural changes at R3 and R2 and the differences in pK_a due to these changes are well reflected in the differences in the chemical shift values of the nitrogen's as well the sum of ^{13}C chemical shifts of the R3 ring. Hence, studying the chemical shifts of the moiety can give us insights into how the pK_a of the molecule will be affected by changes at R3 position.

Correlation between t_{max} and ^{15}N-NMR and ^{13}C-NMR chemical shifts

The time taken to reach maximum concentration in blood, t_{max}, shows excellent correlation with the chemical

shift of the nitrogen in the side-chain -NH (r = 0.94), which can be modeled by the equation:

$t_{max} = 0.213(\pm 0.05)\ (^{15}N\text{-}NH\text{-}) - 7.764(\pm 2.68)$ - Eq.6

n = 4, S.E = 0.47, $R^2 = 0.89$

The chemical shift of the side chain nitrogen varies over a range of 13 ppm in the four molecules studied. There is no correlation between t_{max} and the chemical shifts of other moieties. The correlation between the chemical shift of the side chain nitrogen and t_{max} is significant given that the drugs are hydrolyzed extensively and lose the R2 moiety present very close to this nitrogen. The structural changes in the molecule are reflected in the variations in the chemical shift of the nitrogen and give us an insight into how t_{max} may vary with changes in the structure.

Correlation between half-life and ^{15}N-NMR and ^{13}C-NMR chemical shifts

The elimination half-lives of the ACE inhibitors studied show strong negative correlation with the sum of ^{15}N chemical shifts (r = -0.94). The relationship can be modeled by the following equation:

Half-life = $0.359(\pm 0.091)\ (^{15}N\text{-}Sum) + 81.42(\pm 17.31)$ -Eq.7

n = 4, S.E = 2.19, $R^2 = 0.886$

The half-lives of the molecules also have a negative linear relationship with the substituent at R2 (r = -0.80) as represented in eq.8 and a positive linear relationship (r = 0.71) with the ring R3 substituent as represented in eq.9.

Half-life = $-0.349\ (\pm 0.133)\ (^{13}C\text{-}R2) + 34.78\ (\pm 9.55)$ -Eq.8

n = 6, S.E = 4.40, $R^2 = 0.63$

Half-life = $0.018\ (\pm 0.007)\ (^{13}C\text{-}R3) + 0.068\ (\pm 4.205)$ -Eq.4

n = 6, S.E = 4.39, $R^2 = 0.635$

The ring R1 does not have any correlation with the parameter. Thus, the nitrogen chemical shifts and the chemical shifts of R2 and R3 seem to mirror the structural changes that influence the half-life of ACE Inhibitors.

Correlation between logP and ^{15}N-NMR and ^{13}C-NMR chemical shifts

Lipophilicity is an important property of drugs that determines their ability to permeate and be absorbed by the body. log P is a measure of the lipophilicity of a drug, where P is the partition coefficient of the molecule in water -octan-1-ol system.

The ^{15}N chemical shift of the amino nitrogen was found to have a good negative correlation, r = -0.86, with log P, which is modeled by eq.9. The value of logP increases as the nitrogen is shielded.

$logP = 0.241(\pm 0.099)\ (^{15}N\text{-}NH) + 14.52(\pm 5.096)$ -Eq.9

n = 4, S.E = 0.90, $R^2 = 0.746$

Docking results

The results of the docking studies for all the ligands are summarized in Table IV. The best docking poses for the top three ligands are indicated in Fig. 8. Trandolapril, followed by lisinopril and benazepril exhibited the best activity against angiotensin converting enzyme among the selected ligands. The sum of ^{15}N-NMR chemical shifts of benazepril, enalapril, perindopril and ramipril were compared with the atomic contact energy values and were found to show good correlation (r = 0.83).

Table IV: Screening of ligands based on their atomic contact energy (ACE) values

Name of the ACE inhibitor	Atomic contact Energy (ACE) Values
Benazepril	-125.36
Imidapril	-120.70
Enalapril	-112.73
Perindopril	-101.97
Trandolapril	-152.19
Lisinopril	-131.04
Ramipril	-123.43
Captopril	-182.81

CONCLUSION

Pharmacokinetic and physicochemical properties exhibit linear relationships with NMR chemical shifts of different moieties of ACE inhibitor molecules. The carboxylic group attached to the R2 moiety is involved in the crucial interaction of the inhibitor with the receptor. Similarly, amino nitrogen also forms a hydrogen bond with the enzyme; this interaction is also considered important for inhibition. Structural changes result in variations in pharmacokinetics or physicochemical properties; the same variations are mirrored by NMR chemical shifts of the relevant moieties. In this context, the correlations observed in this study are significant.

Subtle changes in the structures of the molecules within a class can have an effect on pharmacokinetic parameters and clinical decisions. In the absence of experimental data for these parameters, reliable prediction models are required. The relationships between NMR spectral data and pharmacokinetic properties can be applied to develop and improve models for predicting these properties for newer molecules. It will be interesting to study multiple regression models for estimation of pharmacokinetic or physicochemical properties involving both ^{15}N and ^{13}C chemical shifts. However, due to the difficulty in obtaining ^{15}N NMR spectra, the experimental ^{15}N NMR data available are limited. This study is the first report of ^{15}N NMR studies for enalapril, perindopril, ramipril and benazepril and also the first report of ^{15}N NMR studies for any molecules of this class. The lack of reliable prediction programs for ^{15}N-NMR also proves a hindrance in developing larger models based on ^{15}N chemical shifts.

During development of new molecules, spectral data- property relationships can be used to understand how changes in the different functional groups of molecules affect different properties. Insights gathered from such studies can be helpful during the design of new molecules.

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